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DEVELOPMENT OF SPECTROPHOTOMETRIC METHOD FOR ALLOPURINOL IN URINE BASED ON THE DIAZOTIZATION REACTION

(Pembangunan Kaedah Spektrofotometrik untuk Penentuan Allopurinol dalam Air Kencing Berdasarkan Reaksi Diazotisasi)

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Abstract

A simple, rapid, low cost, accurate and sensitive spectrophotometric method for the determination of allopurinol (ALP) in urine was developed. This method is based on the reaction of diazotized sulfanilamide with ALP as coupling agent in an alkaline medium. The resulting intense yellow dye has a maximum absorption at 400 nm and stable for more than 1 hour. The Beer's law limits was obeyed over the concentration range of ALP in aqueous medium from 5.0-50 µg/mL. The molar absorptivity and Sandell's sensitivity were obtained 2.5×10^3 L mol^{-1} cm^{-1} and 0.061 µg cm^{-2} /0,001 absorbance unit, respectively. The optimum reaction conditions and other analytical parameters have also been investigated. The correlation coefficient (r) was obtained 0.999. LOD and LOQ were found 2.2 µg/mL and 7.5 µg/ mL, respectively. The percent recovery and relative standard deviation (RSD) were found 99.18-100.10 % and 0.3-1.3 %, respectively. The proposed method can be applied successfully to assay ALP in urine by standard addition method and can be considered as an alternative method for determination ALP in the urine samples.

Keywords: spectrophotometry, allopurinol, sulfanilamide, diazotization reaction, coupling

Abstrak

Satu kaedah spektrofotometri mudah, cepat, kos efektif, tepat dan sensitif telah dibangunkan untuk penentuan allopurinol (ALP) dalam air kencing. Kaedah ini dibangunkan berdasarkan tindak balas diazotisasi sulfanilamide dengan ALP sebagai ejen gandingan dalam medium beralkali. Pewarna kuning terang yang terhasil mempunyai penyerapan maksimum pada 400 nm dan stabil selama lebih daripada 1 jam. Had hukum Beer dipatuhi pada julat kepekatan ALP dalam medium akueus 5.0-50 µg/mL. Penyerapan molar dan sensitiviti Sandell's masing-masing diperolehi 2.5×10^3 L mol^{-1} cm^{-2} dan 0.061 µg cm^{-2} /0,001 unit serapan. Keadaan tindak balas optimum dan parameter analisis lain juga telah dikaji. Pekali korelasi (r) telah didapati 0.999. LOD dan LOQ didapati masing-masing 2.2 µg/mL dan 7.5 µg/mL. Nilai kebolehulangan dan kejituan sisihan piawai relatif (RSD) didapati masing-masing 99.18-100.10% dan 0.3-1.3%. Kaedah yang dicadangkan boleh digunakan dengan jayanya untuk asai ALP dalam air kencing dengan kaedah tambahan piawai dan boleh dianggap sebagai kaedah alternatif untuk penentuan ALP dalam sampel air kencing.

Kata kunci: spektrofotometri, allopurinol, sulfanilamide, reaksi diazotisasi, gandingan

Introduction

Allopurinol (ALP), chemically known as 1H-pyrazolo [3,4-d] pyrimidin-4-ol shown in Figure1, is used for treatment of gout and hyperuricaemia [1]. It is a xanthine oxidase inhibitor [2,3], which prevents the oxidation of hypoxanthine to xanthine and xanthine to uric acid [4]. Thus results in the reduction of urate and uric acid concentrations in plasma and urine [5]. Synthesis of uric acid plays a role in creating oxidative stress through the formation of oxygen radicals [6]. Physical activity in the form of aerobic exercise can increase free radicals and cause oxidative stress [7]. Reactive oxygen species (ROS) is a very dangerous type of free radicals in the body